Protein Footprinting of HIV Reverse Transcriptase

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A comparison of DNA polymerases displaying a range of processivities suggests that there is a correlation between the degree of processivity and the dimension of template-primer binding cleft formed by the fingers and the thumb subdomains. Time-resolved footprinting will be used to monitor movement of thumb during polymerization. The indirect evidence in support of thumb movement obtained via x-ray crystallography is excellent, and the contacts between HIV-1 RT thumb helices H and I and the template-primer have been mapped with precision. The suggested role of thumb in translocation implies that the thumb contacts with the template-primer are transient and that the thumb must physically move toward the template-primer and then away from it, in concert with the polymerization process. This would also suggest that the thumb contacts template-primer with some periodicity during the polymerization. Changes in solvent accessibility due to changes in protein-nucleic interactions can be monitored both from the standpoint of the nucleic acid and from the standpoint of the protein, depending on the amino acids providing the contacts with the nucleic acid. The thumb domain has three tyrosine residues that will be monitored by protein footprinting and mass-spectrometry. Tyrosine 271, which is near the end on the thumb is likely to modulate its contacts with the nucleic acid during translocation, while tyrosines 310 and 319, which are located near the base of the thumb will provide “control” residues that are unlikely to modulate their interactions during catalysis.

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